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## 2**Extracellular S100 $\beta$ disrupts Bergman glia** 3**morphology and synaptic transmission in** 4**cerebellar Purkinje cells**

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23 **Abstract:** Astrogliosis is a pathological process which affects the  
24 density, morphology and function of astrocytes. It is a common feature  
25 of brain trauma, autoimmune diseases and neurodegeneration  
26 including spinocerebellar ataxia type 1 (SCA1), a poorly understood  
27 neurodegenerative disease. S100 $\beta$  is a Ca<sup>2+</sup> binding protein. In SCA1,  
28 excessive excretion of S100 $\beta$  by reactive astrocytes and its uptake by  
29 Purkinje cells has been demonstrated previously. Under pathological  
30 conditions, excessive extracellular concentration of S100 $\beta$  stimulates  
31 production of proinflammatory cytokines and induces apoptosis. We  
32 modeled astrogliosis by S100 $\beta$  injections into cerebellar cortex in mice.  
33 Injections of S100 $\beta$  led to significant changes in Bergmann glia cortical  
34 organization and affected their processes. S100 $\beta$  also changed  
35 morphology of the Purkinje cells (PCs), causing a significant reduction  
36 of the dendritic length. Moreover, the short-term synaptic plasticity  
37 and depolarization-induced suppression of synaptic transmission, were  
38 disrupted after S100 $\beta$  injections. We speculate that these effects are  
39 due to Ca<sup>2+</sup>-chelating properties of S100 $\beta$  protein. In summary,  
40 exogenous S100 $\beta$  induced astrogliosis in cerebellum could lead to  
41 neuronal dysfunction which resembles a natural neurodegenerative

42 process. We suggest that astrocytes are playing an essential role in  
43 SCA1 pathology and astrocytic S100 $\beta$  is an important contributor to  
44 this process.

45 **Keywords:** astrocytes; S100 $\beta$ ; Purkinje cells; short term plasticity;  
46 Ca<sup>2+</sup> signaling

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## 48 **1. Introduction**

49 Spinocerebellar ataxia type 1 (SCA1) is a progressive  
50 neurodegenerative hereditary disorder which affects mainly the  
51 cerebellum and brainstem. It is caused by a dynamic expansion of CAG  
52 repeats in the N-terminal coding region of *gene* ATXN1 gene on  
53 chromosome 6p23 [1, 2]. In general population, prevalence of SCA1 is  
54 only 1-2 per 100 000, but in specific ethnic groups this ratio varies [3].  
55 SCA1 is characterized by progressive cerebellar dysfunction, dysarthria  
56 and worsening of bulbar functions. Pathological changes include  
57 neuronal loss in the cerebellum, brainstem, and degeneration of  
58 spinocerebellar tracts [4, 5]. Higher cortical functions may also be  
59 affected with symptoms including memory loss, verbal and nonverbal  
60 intellectual deficits [6]. CAG repeats encode amino acid glutamine;  
61 therefore their expansion leads to synthesis of Ataxin 1 with excessive  
62 polyglutamine tract. This affects protein folding and leads to  
63 precipitation as intracellular aggregates [7, 8]. The aggregates also  
64 contain inclusions of ubiquitin, proteasome components and chaperons  
65 [9]. In SCA1 mouse model aggregation occurs later than the first  
66 pathological signs appear and does not correlate with the disease  
67 severity [10]. Accumulation of the mutant protein leads to selective  
68 neurodegeneration in certain regions of the brain and reactive  
69 astrogliosis. Astrogliosis in SCA1 is tightly correlated with the onset  
70 and severity of disease and is not a consequence of neuronal death.  
71 Cvetanovic et al. (2015) described the astrocytic and microglial reaction  
72 in SCA1, using *non-cell selective SCA1 knock-in (Sca1<sup>154Q/2Q</sup>)* and *PC*  
73 *selective B05 SCA1<sup>82Q/2Q</sup>* mouse models. They showed that astrocytes

74and microglia are activated at early stages of SCA1. Expression of  
75protein Ataxin 1 in microglia and astrocytes was not essential for the  
76activation of glia but expression in Purkinje cells was sufficient for it  
77[11, 12].

78In cerebellum, reactive astrogliosis of Bergmann glia may disrupt the  
79spatial distribution of EAAT1 (Excitatory Amino Acids Transporter 1 or  
80Glutamate Aspartate Transporter (GLAST)). This could result in an  
81increase in extracellular glutamate concentrations and toxicity via  
82NMDA-receptors [12, 13]. In addition, activated astrocytes and  
83microglia are able to release various proinflammatory molecules. Some  
84of them, such as Tumor Necrosis Factor  $\alpha$  (TNF $\alpha$ ), interleukin-6 (IL6)  
85and monocyte chemoattractant protein-1 (MCP-1) have been implicated  
86in neurodegeneration and negatively affect the function and survival of  
87neurons [14–19]. S100 $\beta$  protein is one of the S100 Ca<sup>2+</sup>-binding proteins  
88of the S100 group which includes nearly 20 members [20]. S100  
89proteins form homo- and heterodimers and are able to chelate not only  
90Ca<sup>2+</sup>, but also Zn<sup>2+</sup> and Cu<sup>2+</sup> [21]. Binding of the ions changes the  
91confirmation of S100 and alters their affinity to different ligands (more  
92than 90 potential targets known currently) [20–23].

93Here we model astrogliosis in cerebellum, such as seen in SCA1, by the  
94intracortical injections of S100 $\beta$ . We demonstrate a significant negative  
95impact of this astrocytic protein to the PCs morphology and synaptic  
96transmission in the parallel fibre-Purkinje cell (PF-PC) synapse.  
97Moreover, we demonstrate that short term synaptic plasticity, the  
98depolarization-induced suppression of excitation (DSE) is disrupted by  
99S100 $\beta$ . We speculate that these effects could be attributed to the Ca<sup>2+</sup>-  
100chelating properties of S100 $\beta$ .

## 1012. Materials and Methods

102 All procedures for the care and treatment of animals were carried  
103out according to the Krasnoyarsk State Medical University and Russian  
104public standard (33215-2014) regulations and approved by the local  
105ethical committee. Every effort was made to minimize animal suffering  
106and to reduce the number of animals used in this study. 12 weeks old

107CD-1 IGS WT mice (Charles River Laboratories) were used in this study.  
1084 weeks old non-cell selective SCA1<sup>154Q/2Q</sup> knock-in (SCA1 KI) mice with  
109C57BL/6J background used in this work [24]. Experiments with SCA1 KI  
110mice were generated in Gunma University (Japan) in laboratory of  
111Neurophysiology and Neuronal Repair led by Professor H. Hirai. These  
112were mice kindly provided by Dr. Hidehiro Mizusawa (Tokyo Medical  
113and Dental University). Animals were kept on a 12-h light/dark cycle  
114with free access to food and water.

115

### 1162.1. *Drugs and reagents*

117 All reagents for electrophysiological experiments were from Sigma  
118Aldrich.

119 Recombinant mouse S100 Calcium Binding Protein B (S100 $\beta$ ) (Cat.  
120No. APA567Mu01) was obtained from Cloud-Clone Corp. Concentrated  
121stock solution of S100 $\beta$  was initially prepared and diluted in  
122physiological saline (PBS) to a final concentration before use.  
123Fluorocitrate (FC) was used as barium salt (Cat. F9634, Sigma Aldrich)  
124as described previously (Paulsen et al., 1987). (3S)-3-[[3-[[4-  
125(trifluoromethyl)benzoyl]amino]phenyl]methoxy]-L-aspartic acid (TFB-  
126TBOA) (Cat. No. 2532) was obtained from Tocris. Concentrated stock  
127solutions of FC and TBOA were initially prepared and diluted in artificial  
128spinocerebellar fluid (ACSF) to their final concentrations before use.

### 1292.2. *S100 $\beta$ injections*

130 12-week-old (P90-P100) WT CD1 mice were anaesthetized by  
131intraperitoneal injection of chloral hydrate solution (400mg/kg of body  
132weight). 2.5  $\mu$ l of 50  $\mu$ M S100 $\beta$  in phosphate buffered saline (PBS) or  
133PBS was stereotactically injected into the cortex of cerebellar vermis  
134(lobule VI) using 10  $\mu$ l Hamilton syringe. To reach the injection point  
135in the vermis we used the coordinates relative to bregma: AP: -2.5 mm,  
136ML: 0 mm, DV: 2mm. Mice were used 24 hours after the injection.

### 1372.3. *Electrophysiology*

138 Cerebellar slices (250  $\mu\text{m}$  thick) were prepared, and whole-cell  
139 recordings were conducted as described previously [25]. Briefly, mice  
140 were deeply anesthetized by intraperitoneal injection of chloral hydrate  
141 (400 mg/kg of body weight) and killed by decapitation. The brain was  
142 quickly dissected and placed for one minute in an ice-cold Ringer's  
143 solution containing: 234  $\text{mM}$  sucrose, 26  $\text{mM}$   $\text{NaHCO}_3$ , 2.5  $\text{mM}$   $\text{KCl}$ ,  
144 1.25  $\text{mM}$   $\text{NaH}_2\text{PO}_4$ , 11  $\text{mM}$  glucose, 10  $\text{mM}$   $\text{MgSO}_4$ , and 0.5  $\text{mM}$   $\text{CaCl}_2$   
145 0.5; pH 7.4, continuously oxygenated with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ .  
146 Parasagittal slices of cerebellar vermis were made using a microslicer  
147 (Thermo Scientific; Microtom CU65). The slices were maintained in an  
148 extracellular solution containing: 125  $\text{mM}$   $\text{NaCl}$ , 2.5  $\text{mM}$   $\text{KCl}$ , 2  $\text{mM}$   
149  $\text{CaCl}_2$ , 1  $\text{mM}$   $\text{MgCl}_2$ , 1.25  $\text{mM}$   $\text{NaH}_2\text{PO}_4$ , 26  $\text{mM}$   $\text{NaHCO}_3$ , 10  $\text{mM}$  D-  
150 glucose, and 0.05-0.1  $\text{mM}$  picrotoxin bubbled by 95%  $\text{O}_2$  /5%  $\text{CO}_2$  gas  
151 mix at room temperature for 1h before starting the electrophysiological  
152 experiments. For current clamp whole-cell recordings from Purkinje  
153 cells (PCs) we used K-gluconate-based intracellular solution containing:  
154 130  $\text{mM}$  K-gluconate, 4  $\text{mM}$   $\text{KCl}$ , 20  $\text{mM}$  HEPES, 1  $\text{mM}$   $\text{MgCl}_2$ , 4  $\text{mM}$   
155  $\text{MgATP}$ , 1  $\text{mM}$   $\text{NaGTP}$ , 0.4  $\text{mM}$  EGTA (pH 7.3 adjusted with  $\text{KOH}$ ). For  
156 voltage clamp whole-cell recordings from Purkinje cells (PCs) we used  
157 intracellular solution containing: 140  $\text{mM}$  Cs-gluconate, 8  $\text{mM}$   $\text{KCl}$ , 10  
158  $\text{mM}$  HEPES, 1  $\text{mM}$   $\text{MgCl}_2$ , 2  $\text{mM}$   $\text{MgATP}$ , 0.4  $\text{mM}$   $\text{NaGTP}$ , 0.4  $\text{mM}$   
159 EGTA (pH 7.3 adjusted with  $\text{CsOH}$ ). Passive electrical properties of the  
160 PCs were estimated using averaged traces of  $\sim 10$  current responses to  
161 hyperpolarising voltage pulses (from -70 to -80 mV, 200 ms duration).  
162 Fast capacitance component was automatically compensated; signal  
163 was sampled at 50 kHz and low-pass filtered at 10 kHz. No correction  
164 was made for liquid junction potentials. Analysis of electrophysiological  
165 data was performed using pClamp10 (Molecular Devices), Patchmaster  
166 software (HEKA), and Clampfit 10.5 (Axon instruments).

167 PCs were voltage-clamped at -70 mV to record PF EPSCs. Selective  
168 stimulation of PFs was confirmed by paired-pulse facilitation of EPSC  
169 amplitudes (at a 50-ms interstimulus interval).

170 To examine depolarization induced suppression of excitation (DSE),  
171 PF EPSCs were recorded every 3 s. After monitoring basal PF EPSCs

172for 1 min, a single depolarizing pulse (5s from -70 to 0 mV) was applied  
173to the recorded PC. This opens the voltage gated  $\text{Ca}^{2+}$  channels (VGCC)  
174and releases endocannabinoids which presynaptically decrease  
175glutamate release and suppress amplitude of PF EPSC [26]. Amplitudes  
176of subsequent PF EPSCs were normalized to the mean value of 12  
177responses evoked before the induction of DSE.

#### 1782.4. Immunohistochemistry

179 For immunohistochemistry (IHC), anesthetized mice were perfused  
180transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer.  
181The brain was postfixed in the same fixative overnight. The cerebellar  
182vermis was cut into 50- $\mu\text{m}$  sagittal sections. The sections were treated  
183with rabbit monoclonal anti-calbindin D-28 k (1:500, Cloud Clone Corp.,  
184China), chicken polyclonal anti-GFAP antibodies (1:1000, Abcam, UK),  
185rabbit polyclonal anti-S100 $\beta$  (1:1000, Abcam, UK). Secondary  
186antibodies were Alexa Fluor 514-conjugated donkey anti-rabbit IgG  
187(1:1,000, Life Technologies), Alexa Fluor 647-conjugated donkey anti-  
188chicken IgG (1:1,000, Life Technologies) and Alexa Fluor 488-  
189conjugated donkey anti-rabbit IgG (1:1,000, Life Technologies).  
190Antibodies were dissolved in PBS solution containing 2% (v/v) normal  
191donkey serum, 0.1% (v/v) Triton X-100, and 0.05%  $\text{NaN}_3$ .

192

#### 1932.5 Confocal microscopy and morphometric analysis

194

195 Fluorescent images were obtained using confocal microscope *and*  
196*original software of* Olympus, Fluoview, FV10i (Japan). Images were  
197recorded as Z-stacks of 0.25  $\mu\text{m}$  thickness with *x10 lense*, numerical  
198aperture of 1.0, *zoom x6, at 1024x1024 resolution*. In all groups the  
199cerebellar lobes 6 and 7 of the vermis cerebellum were used for  
200comparison (Fig. 1). For double labeling, images from the same  
201confocal plane were taken. Alexa Fluor 647 signal (blue) was artificially  
202changed to red color to show S100 $\beta$ /GFAP colocalization in merge  
203microphotographs (yellow) (Fig. 1 and 2).

204 Thickness and number of Bergmann glia processes were measured on  
 205 confocal images of sagittal cerebellar slices. The number of radial glial  
 206 processes for 100  $\mu\text{m}$  of in the molecular layer (Sup. Fig. 1A) were  
 207 counted. The same 100  $\mu\text{m}$  line intensity profile was used to obtain the  
 208 distribution of GFAP fluorescence, using the original software of the  
 209 Olympus confocal microscope. Each glial process was shown as peak of  
 210 GFAP/Alexa 647 fluorescence intensity. We counted the averaged  
 211 thickness of these processes in each image. To avoid false positive  
 212 enhancement of processes thickness we used cut-off threshold for  
 213 recognition of the GFAP signal set to 10% of the maximal fluorescence  
 214 intensity. To count the number of Bergmann glia cells, we measured  
 215 anti-S100 $\beta$  positive circle- and oval-shaped signals in Purkinje cell layer.  
 216 To avoid over- or underestimation, the glial cell numbers were  
 217 calculated by ceiling the ratio  $n = \lceil \frac{d}{d'} \rceil$ , where n is the cell number, d is  
 218 the length of S100 $\beta$  positive signal in  $\mu\text{m}$  and  $d' = 15 \mu\text{m}$  which we took for  
 219 characteristic diameter of an astrocyte. The approximate length of the  
 220 dendrites of the Purkinje cells was estimated from overall thickness of  
 221 the molecular layer (Sup. Fig. 1B), visualized using anti-calbindin/Alexa  
 222 488 staining.

223

## 224 2.6. Sholl analysis of Bergmann glia cells

225 Quantitative morphological analysis was performed in the three-  
 226 dimensional (3D) mode. Using a confocal laser scanning microscope  
 227 (Olympus, Fluoview, FV10i) anti-GFAP-labeled Bergmann glia was  
 228 scanned in Z-stacks (80–150 consecutive focal planes at 0.25  $\mu\text{m}$   
 229 interval). For Sholl analysis Z-stacks images of soma and processes  
 230 of Bergmann glia were traced on focal planes using ImageJ  
 231 software. We used the Sholl method of concentric circles using an  
 232 ImageJ regime (set of nested concentric spheres is centered on the  
 233 cell body, and the spheres increase in size by 10  $\mu\text{m}$  radius) [27]. The  
 234 results of Sholl analysis showed length of processes and the number  
 235 of intersections per 10  $\mu\text{m}$ .

## 2362.7. Statistical and mathematical analysis

237 Pooled data are expressed as the mean  $\pm$  SEM. Statistical analyses  
238of differences between the groups were performed using the unpaired t-  
239test and Mann-Whitney U test. The influence of FC and TBOA on the  
240EPSC recovery was estimated with one-way ANOVA test. Differences  
241were considered significant at  $p < 0.05$ .

242

243 We estimated the dendritic and somatic capacitance by optimization  
244of the two-term exponential series to the current response curve to the

245voltage step  $V_{step} = 10$  mV [28]:

$$246 \quad I_{clamp}(t) = V_{step} \left( \frac{1}{R_{ss}} - A_d \tau_d e^{-\frac{t}{\tau_d}} - A_s \tau_s e^{-\frac{t}{\tau_s}} \right).$$

$$247 \quad I_{clamp}(t) = V_{step} \left( \frac{1}{R_{ss}} - A_d \tau_d e^{-t/\tau_d} - A_s \tau_s e^{-t/\tau_s} \right)$$

248to find the time constants  $\tau_i$ . Here  $R_{ss} = 4$  M $\Omega$  is the input  
249resistance and  $A_i$  are the free parameters. Indices d and s stand for  
250dendritic and somatic components respectively. The resulting

251capacitance was then calculated as  $C_i = \frac{\tau_i}{R_m}$  ( $i = d, s$ ).  $R_m$

252 $R_m$  is the membrane resistance. Optimization was made in ClampFit  
25310.7 software.

254

255 DSE was analyzed using dual exponential waveform equation (1).

$$256 \quad DSE(t) = 100 + A \left( e^{\frac{-t}{\tau_1}} - e^{\frac{-t}{\tau_2}} \right) \left( A = \frac{100 a \tau_1 \tau_2}{\tau_1 - \tau_2} \right) \quad (1)$$

257 This curve is convenient for the prediction of the conduction  
258changes in synapses [29]. It contains the parameters for both decay  
259and recovery of EPSC separately during the DSE protocol. This model



260 was fitted to the experimental data by the Nelder-Mead minimization of  
261 the sum of squared residuals to find “ $A$ ” – the maximum EPSC decrease  
262 in percent of initial level, “ $\tau_1$ ” and “ $\tau_2$ ” – the half-times for the EPSC to  
263 reach the minimum and to recover to the initial 100%, respectively. The  
264 parametric bootstrap technique was used to obtain the 95% confidence  
265 intervals for the parameters  $A$ ,  $\tau_1$ ,  $\tau_2$ . This analysis was performed using  
266 Python 3 package.

## 2673. Results

### 2683.1. *Exogenous S100 $\beta$ alters morphology of Bergmann glia*

269 2.5 $\mu$ l of 50 $\mu$ M S100 $\beta$  were injected in the cerebellar cortex to  
270 induce astrogliosis. 24 hours later widespread distribution of  
271 S100 $\beta$  and increased GFAP expression in cerebellar cortex were  
272 evident in lobules IV-VII (Fig. 1C compare to Fig. 1A, note that in  
273 the normal brain immunofluorescent GFAP was poorly visible).  
274 In microphotographs of S100 $\beta$  injected areas prominent GFAP  
275 positive striation was evident (contrast to PBS injected areas,  
276 Fig. 1B and D). To analyze the morphology of Bergmann glia we  
277 examined the number and thickness of anti-GFAP-positive glial  
278 processes in the central part of molecular layer per 100 $\mu$ m using  
279 line profile function (Sup. Fig. 1A and Fig. 2A and B). The  
280 average cross-section of Bergmann glia processes in S100 $\beta$   
281 injected mice was increased to  $3.6 \pm 0.1$   $\mu$ m, (364 processes from  
282 17 areas of 3 mice) vs  $2.8 \pm 0.1$   $\mu$ m, (358 processes from 11 areas  
283 of 3 mice) in PBS injected mice,  $p = 1.25 \times 10^{-13}$ , unpaired t-test  
284 (Fig. 2C). The number of processes per 100 $\mu$ m longitudinal  
285 length of molecular layer in S100 $\beta$  injected animals was  
286 significantly decreased compared to PBS injected animals  
287 ( $21.4 \pm 2.0$  vs  $32.6 \pm 3.3$ ,  $p = 0.013$ , t-test; Fig. 2D). The density of  
288 Bergmann glia processes was also decreased in S100 $\beta$  injected  
289 areas. We also measured the fraction of "GFAP-negative" space  
290 in the central part of molecular layer per 100 $\mu$ m using the same  
291 line profile function. In S100 $\beta$  injected areas (17 areas of 3 mice)  
292 it was increased to  $24.0 \pm 4.5\%$  compared with  $8.1 \pm 1.6\%$  in PBS-  
293 injected areas (11 areas of 3 mice,  $p = 0.015$ ; unpaired t-test;  
294 Fig. 2E). Low Bergmann glia processes density is mainly due to  
295 the loss of some of these cells. Indeed, the number of anti-S100 $\beta$ -  
296 labeled cell bodies per 100 $\mu$ m longitudinal length of Purkinje cell  
297 layer in S100 $\beta$  injected animals was significantly decreased in  
298 comparison to PBS injected animals ( $9.1 \pm 0.4$  vs  $10.9 \pm 0.5$ ,  $p =$   
299  $0.007$ , unpaired t-test; Fig. 2F).

300 Next, we studied single astrocyte morphology using Sholl  
301 analysis (Sholl, 1953). On images of digitally traced Bergmann  
302 glia processes (Fig. 3A) we analyzed the maximum number of  
303 these processes per cell. This number was not changed in S100 $\beta$   
304 injected areas ( $4.9 \pm 1.2$ , n=12 from 3 animals) in comparison to  
305 PBS injected areas ( $4.8 \pm 1.7$ , n=10 from 3 animals, p=0.648;  
306 Mann-Whitney U test; Fig. 3B). Sholl analysis revealed an  
307 increase in the density of proximal processes in Bergmann glia  
308 after S100 $\beta$  injections. Within 10 $\mu$ m from soma in S100 $\beta$  injected  
309 areas (12 areas of 3 mice) Bergmann glia had  $3.3 \pm 0.3$  processes,  
310 while in PBS injected areas was  $1.8 \pm 0.2$  (p = 0.0002; 11 areas of  
311 3 mice, Mann-Whitney U test; Fig. 3C.)

312 These data show that excessive extracellular S100 $\beta$  protein in the  
313 cerebellar cortex leads to significant changes in Bergmann glia  
314 morphology.

### 315 3.2. Extracellular S100 $\beta$ alters morphology of Purkinje cells

316 As shown above, S100 $\beta$  affects glia and it is well-known that  
317 disturbances in glia may lead to neuronal degeneration [30–32]. In  
318 addition, S100 $\beta$  could have a direct effect on Purkinje cells. We  
319 examined the effect of S100 $\beta$  on morphology of these neurons using  
320 IHC and their physiological state using patch clamp. To estimate the  
321 approximate dendritic length of PCs cells were visualized by anti-  
322 calbindin staining (Fig. 4A) and measured the thickness of the  
323 molecular layer (Sup. Fig. 1B). S100 $\beta$  injections reduced it to  $120.0 \pm 5.8$   
324  $\mu$ m (n=12 areas from 3 mice) compared to  $150.7 \pm 6.3$   $\mu$ m, n = 14 areas  
325 from 3 mice in PBS injected mice (p=0.002, t-test; Fig. 4B).

326 Using patch clamp we estimated capacitance of dendrites and soma  
327 after subtraction of slow capacitance component from the total  
328 capacitance of PCs (see materials and methods). Slow component  
329 reflects predominantly the size of neuronal dendrites. We found a  
330 significant difference between the two groups. The capacitances of PCs  
331 dendrites in S100 $\beta$  injected mice were  $359.4 \pm 37.5$  pF (n=33 cells from  
332 8 mice) and  $513.5 \pm 27.1$  pF (n = 52 cells from 10 mice) in PBS injected

333group. ( $p=0,002$ , t-test; Fig. 3B). The capacitances of PCs soma in  
334S100 $\beta$  injected mice were  $34.6\pm4.4$ pF (the same cells) and  $61.7\pm5.6$ pF  
335(the same cells) in PBS injected group. ( $p=0,0003$ , t-test; Fig. 3C).

336 These data indicate that excessive extracellular S100 $\beta$  affects PCs  
337morphology, leading to the collapse of the soma and dendrites.

### 3383.3. *Extracellular S100 $\beta$ alters synaptic transmission in PFs and PCs*

339 Astrocytes control removal of glutamate from the presynaptic space  
340[33–36]. Moreover, astrocytic secretion of S100 $\beta$  protein into the  
341intercellular space leads to endocytosis of this protein by neurons and  
342evokes various effects such as chelation of cytoplasmic Ca<sup>2+</sup> [20]. For  
343this reason, we tested whether elevated extracellular S100 $\beta$  affects  
344synaptic transmission in PF-PCs synapses. S100 $\beta$  did not change the  
345PF-EPSCs amplitude (Sup. Fig. 2A). We suspected that S100 $\beta$  will affect  
346processes highly dependent on Ca<sup>2+</sup> release, such as presynaptic  
347glutamate secretion. However, we did not see significant differences  
348between PPF ratio in PF-PC synapses of S100 $\beta$  and PBS injected mice.  
349The PPF ratio in S100 $\beta$  injected mice was  $1.8\pm0.4$ ,  $n=35$  cells from 8  
350mice vs  $1.9\pm0.1$ ,  $n=39$  cells from 9 mice in PBS injected mice  
351( $p=0.722$ , t-test; Fig. 5A). However, S100 $\beta$  dramatically affected the  
352kinetics of PF-EPSCs. While there was no statistically significant  
353difference in the PF-EPSCs amplitude in mice injected with S100 $\beta$  and  
354PBS (Sup. Fig. 2A), the rise time of PF-EPSC in S100 $\beta$  injected mice  
355was prolonged to  $2.7\pm0.1$ ms ( $n=35$  cells from 8 mice), compared to  
356 $2.3\pm0.1$ ms ( $n=36$  cells from 9 mice) in PBS injected mice ( $p=0.028$ , t-  
357test; Fig. 5B).

358 To control for the potential effect of surgery per se, we analyzed this  
359parameter in sliced from naïve mice. No difference was found between  
360naïve and **PBS** injected groups (Sup. Fig. 3).

361 *In addition the decay time of PF-EPSC in S100 $\beta$  injected mice was*  
362 $17.1\pm1.5$ ms ( $n=30$  cells from 8 mice), *while it increased to*  $21.6\pm1.5$ ms  
363( $n=37$  cells from 9 mice) *in PBS injected mice* ( $p=0.04$ , t-test; Fig.  
3645C).

365 These results demonstrate that the excessive extracellular  
366 accumulation S100 $\beta$  protein mainly affects kinetics of PF-EPSC, which  
367 most likely reflects changes glutamate removal from the synaptic cleft.

368

369 *3.4. Similarities in changes in synaptic transmission in S100 $\beta$ -*  
370 *injected mice and Ataxin1 mutant animals.*

371 To look for similarities between SCA1 and consequences of S100 $\beta$   
372 injections we used KI mice with non-cell selective expression of mutant  
373 Ataxin 1 [24]. Mice were used at 3 weeks of age which corresponds to  
374 the early stage of neurodegenerative process. There was no statistically  
375 significant difference in the PF-EPSCs amplitude recorded in the PCs of  
376 SCA1 KI and WT mice (Sup. Fig. 2B). The PPF ratio in SCA1 KI mice  
377 was  $2.1 \pm 0.1$ ,  $n=10$  cells from 3 mice and  $1.8 \pm 0.1$ ,  $n = 8$  cells from 3  
378 mice in WT mice ( $p=0.012$ , unpaired t-test; Fig. 6A). PCs from SCA1 KI  
379 mice have altered kinetics of PF-EPSCs. The average rise time of PF-  
380 EPSC in SCA1 KI mice significantly increase to  $3.0 \pm 0.2$ ms ( $n=10$  cells  
381 from 3 mice), compared to WT mice ( $2.3 \pm 0.2$ ms;  $n = 8$  cells from 3  
382 mice;  $p=0.038$ , unpaired t-test; Fig. 6B). The differences in decay time  
383 of PF-EPSC between SCA1 KI mice and WT were not significant  
384 ( $18.4 \pm 2.1$ ms,  $n=10$  cells from 3 mice vs.  $15.9 \pm 3.3$ ms,  $n = 8$  cells from 3  
385 mice;  $p=0.379$ , unpaired t-test; Fig. 6C).

386 *3.5. Extracellular S100 $\beta$  alters endocannabinoid-dependent short term*  
387 *plasticity in PF-PC synapses*

388 As mentioned above, we expected that S100 $\beta$  could affect processes  
389 which are known to depend on the cytosolic concentration of Ca<sup>2+</sup>. One  
390 of such processes is DSE, which is evoked by the membrane  
391 depolarization. Depolarization leads to opening of voltage-gated  
392 calcium channels (VGCC) and an increase in the intracellular Ca<sup>2+</sup>. Ca<sup>2+</sup>  
393 triggers endocannabinoid release from the postsynaptic cell with  
394 consecutive activation of CB1 receptors on the presynaptic terminal,  
395 leading to a reduction in release of glutamate. We examined dynamic of  
396 PF-EPSC amplitude after 5 sec of depolarization from -70 to 0 mV. The  
397 stimulus intensity was adjusted to reach EPSC amplitude of

398 approximately 150 pA before DSE induction. In control mice DSE  
399 protocol reduced EPSC by  $67.3 \pm 3.5\%$  ( $n = 11$  cells from 4 mice) which  
400 was similar to that in mice pre-injected with S100 $\beta$  ( $69.7 \pm 4.7\%$ ,  $n = 12$   
401 cells from 4 mice,  $p = 0.975$ , unpaired t-test; Fig. 7A). However the  
402 recovery of the PCs amplitude was significantly faster in S100 $\beta$  injected  
403 mice. 50 sec after the challenge amplitude returned to  $93.6 \pm 2.8\%$  of  
404 control, compared to  $83.9 \pm 2.7\%$  in PBS injected mice ( $p = 0.03$ ,  
405 unpaired t-test; Fig. 7A and B).

406 The double waveform model fit (1) confirmed slowing of the  
407 recovery kinetics by S100 $\beta$ . The amplitude reduction in the DSE  
408 protocol was not significantly different in S100 $\beta$  injected mice  
409 compared to PBS group:  $45.5$  ( $30.6$ ,  $63.6$ )% vs  $38.1$  ( $31.8$ ,  $44.6$ )%  
410 respectively. Using the fitting protocol we calculate that the half-time  
411 for the recovery of the parameter ( $\tau_2$ ) is significantly smaller in S100 $\beta$   
412 injected mice with  $29.7$  ( $21.0$ ,  $47.2$ ) sec in comparison with  $64.5$  ( $52.0$ ,  
413  $85.1$ ) sec in PBS group,  $p < 0.05$ .

414 These results demonstrate that excessive extracellular S100 $\beta$   
415 protein negatively affects DSE.

416

### 417 3.6. Effects of FC on PF-PC transmission and endocannabinoid short 418 term plasticity in PF-PC synapses

419 FC inhibits astrocytic metabolism and deprives these cells of energy,  
420 this leading to an array of repercussions which ultimately undermine  
421 functions of these cells [37]. Application of FC led to a strong  
422 depression of PF-PC excitatory transmission, irrespective of whether  
423 the tissue was exposed to S100 $\beta$  or not (Fig 8A and B). After 10 min  
424 application the amplitude of PF-EPSC in S100 $\beta$  injected mice decreased  
425 up to  $57.7 \pm 9.0\%$  of control ( $n = 7$  cells from 4 mice,  $p = 0.006$ , paired t-  
426 test) and in PBS injected mice up to  $64.4 \pm 9.9\%$  of control ( $n = 7$  cells  
427 from 4 mice,  $p = 0.014$ , paired t-test) (Fig. 8A and B). 10 min after FC  
428 application averaged PF-EPSCs in S100 $\beta$  and PBS injected groups were  
429 not different (unpaired t-test,  $p = 0.65$ ). The rise time of PF-EPSC in PBS  
430 and S100 $\beta$  injected mice increased after FC treatment. In PBS group it  
431 increased from  $2.3 \pm 0.2$  ms to  $2.7 \pm 0.2$  ms ( $n = 12$  cells from 4 mice,

432  $p=0.003$ , paired t-test; Fig. 8C) while in S100 $\beta$  group it increased from  
433  $2.4\pm0.3$ ms to  $3.5\pm0.6$ ms ( $n = 10$  cells from 3 mice,  $p=0.003$ , paired t-  
434 test; Fig. 8C).

435 It was shown previously that astrocytes also contain CB1 receptors  
436 and could modulate the synaptic plasticity [38]. CB1 receptors in  
437 astrocytes are coupled to  $G_{q/11}$ -proteins and trigger PLC activation [39]  
438 and release such gliotransmitters as glutamate, ATP or d-serine [40].  
439 This phenomenon is SNARE-dependent and highly sensitive to ATP  
440 concentration which should be reduced by FC [41, 42]. However FC did  
441 not affect expression of DSE. After FC application in PBS injected mice  
442 DSE protocol reduced EPSC by  $56.8\pm8.7\%$  ( $n = 9$  cells from 4 mice)  
443 which was not statistically different from what registered without FC in  
444 S100 $\beta$ -injected mice (Fig. 8E) ( $p=0.317$ , unpaired t-test). However, this  
445 comparison is compromised by the direct impact of FC on PF-induced  
446 EPSCs and should be interpreted with care.

447 The double waveform model fit the changes of PF-EPSC amplitudes  
448 after depolarization pulse and DSE initiation. Maximum amplitudes  
449 reduction was  $51.92$  ( $41.67$ ,  $81.41$ )% and  $46.23$  ( $33.28$ ,  $75.49$ )% for PBS  
450 and S100 $\beta$  respectively.

451 However the recovery of the PCs amplitude was significantly slower  
452 in FC treated PCs in slices from PBS-treated mice (Fig. 8F). 50 sec after  
453 the challenge, it returned to  $67.5\pm5.4\%$  of control, compared to  
454 untreated slices where it recovered to  $83.9\pm2.7\%$ ,  $p = 0.025$ , unpaired  
455 t-test; Fig. 8D-F. However, FC had hardly any effect on DSE protocol in  
456 S100 $\beta$  injected mice where recovery of the PCs amplitude was  
457  $81.4\pm7.6\%$  of PF-EPSC amplitude at 50 sec post challenge in FC vs  
458  $93.6\pm2.8\%$  without FC;  $p = 0.181$ , unpaired t-test (Fig. 8D-F).

459 Double waveform model fit also did not reveal significant changes  
460 after the FC treatment.

461

462 *3.7. Slowdown of glutamate uptake in Bergmann glia by TBOA leads to*  
463 *alteration of PF-EPSC kinetic but does not change endocannabinoid*  
464 *short term plasticity in PF-PC synapses*

90% of all glutamate uptakes in PF-PC synapses is due to excitatory amino acid transporters EAAT1 and EAAT2, located on the membranes of astrocytes [43, 44]. We suspected that shortening of PF-EPSC decay time after S100 $\beta$  injection was due to facilitation of glutamate reuptake through EAATs (Fig. 5C). 500 $\mu$ M TBOA significantly increased decay time of PF-EPSC in PBS and S100 $\beta$  injected mice. In PBS injected mice decay time changed from  $30.0 \pm 4.4$  to  $39.2 \pm 6.3$  msec (n=16 cells from 4 mice,  $p=0.039$  paired t-test, Fig. 9A). Note that before TBOA application decay constants were different between S100 $\beta$  and PBS injected groups ( $p=0.044$ , one way ANOVA) while after TBOA they reached approximately same values ( $p=0.64$ , one way ANOVA, Fig. 9A). In S100 $\beta$  injected mice increase was even more dramatic, from  $19.2 \pm 2.7$  ms to  $34.6 \pm 7.1$  ms (n=10 cells from 3 mice,  $p=0.031$  paired t-test). Application of nonselective EAAT blocker DL-TBOA 500 $\mu$ M did not significantly change PF-EPSC amplitudes and rise time in cerebellum of both PBS and S100 $\beta$  injected mice (data not shown). TBOA did not affect PPF ratio of PF-EPSC in PBS injected mice ( $1.86 \pm 0.1$  vs  $1.84 \pm 0.1$ ; n=16 cells from 4 mice,  $p=0.673$  paired t-test), while in S100 $\beta$  injected mice it resulted in a slight but significant PPF ratio reduction from  $1.9 \pm 0.2$  to  $1.76 \pm 0.1$  (n=10 cells from 3 mice,  $p=0.049$  paired t-test, Fig. 9B).

We examined endocannabinoid short term plasticity in PF-PC synapses after treatment of cerebellar slices with 500 $\mu$ M TBOA. In PBS injected mice DSE protocol was not affected by TBOA. EPSC were reduced by  $59.2 \pm 5.4\%$ ; n = 15 cells from 4 mice) which was not statistically different from that before application ( $56.8 \pm 5.7\%$ ,  $p = 0.524$ , paired t-test). Also, the recovery of the PCs amplitude was not affected by TBOA (Fig. 9C and D). Neither did TBOA affect the outcome of DSE protocol in S100 $\beta$  injected mice ( $74.8 \pm 9.6\%$  reduction in TBOA, n = 11 cells from 4 mice vs  $73.6 \pm 8.8\%$  without TBOA,  $p = 0.789$ , paired t-test). Also the recovery of the PCs amplitude was unaffected by TBOA. The double waveform model yielded in the PF-EPSC amplitudes reduction during DSE as  $45.71$  (37.39, 56.75) % and  $32.11$  (14.73, 75.00) % for PBS and S100 $\beta$  cells respectively. The restoration half-time  $\tau_2$  was  $69.28$  (53.08,



49992.12) sec for PBS cells and 46.72 (18.68, 113.44) sec for S100 $\beta$  cells.  
500These results confirm that TBOA did not affect the outcome of the DSE  
501(Fig. 9 C and D).

502These results demonstrate that the slowdown of glutamate uptake  
503predictably affects PF-EPSC decay time and does not change DSE.

#### 5044. Discussion

505 It was shown previously that activated astrocytes secrete large  
506 amounts of S100 $\beta$  [11, 18]. PCs absorb glial S100 $\beta$  in cytoplasmic  
507 vacuoles, which leads to changes in their morphology and degeneration  
508 [19, 45]. In SCA1 B05 tg mouse model, formation of S100 $\beta$ -containing  
509 cytoplasmic vacuoles precedes accumulation of the mutant Ataxin 1 and  
510 appearance of the ataxic phenotype [11]. Downregulation of S100 $\beta$   
511 rescues the neurological deficit; therefore, it has been argued that this  
512 protein plays the central role in neurodegeneration [46]. We attempted  
513 to mimic some of the features of SCA1 by injections of S100 $\beta$ . Mouse  
514 S100 $\beta$  was used in order to avoid an immune reaction to a foreign  
515 antigen. Using immunohistochemistry we found that the area of S100 $\beta$   
516 deposits spread much further than the actual site of injection and  
517 covered 2-3 lobes of the cerebellum. The maximal  
518 immunohistochemical signal was seen in lobes 5-7 (Fig. 1C). For  
519 imaging we adjusted the brightness of S100 $\beta$ /Alexa 488 fluorescence  
520 signal so that to be able to image high concentration S100 $\beta$  without  
521 saturation of the system. For this reason, the fluorescence of the  
522 endogenous S100 $\beta$  in control mice appears low (Fig. 1A and B). To  
523 avoid false positive results when measuring the BG and PC morphology,  
524 we used the lobes 6 and 7 which were not directly affected by the  
525 injection in all experiments. The excessive amount of S100 $\beta$  altered the  
526 morphology of Bergmann glia. Reduction of Bergmann glia cell number  
527 (Fig. 2B and F) and processes (Fig. 2D) was accompanied by thickening  
528 of the processes (Fig. 2C) and sprouting of new processes in presomatic  
529 areas (Fig. 3A and C).

530 The thickness of molecular layer correlates with the length of PC  
531 dendritic tree [47]. To examine the approximate length of PCs dendrites  
532 we measured thickness of molecular layer and found that it was  
533 significantly reduced by S100 $\beta$  (Fig. 4A and B). Accordingly, soma and  
534 dendritic capacitance of PCs also changed significantly (Fig. 4C).

535 It is interesting that S100 $\beta$  may modulate sodium channels in  
536 neurons via Ca<sup>2+</sup> chelation which affects neuronal sodium channels [48]

537which may result in bursting. Kolta with coauthors showed that  $\text{Ca}^{2+}$   
538chelation by S100 $\beta$  in intercellular space leads to  $\text{Na}^+$  currents  
539enhancement and make them fire action potentials in bursts, rather  
540than single AP. It is very likely open NMDA receptors and bring more  
541 $\text{Ca}^{2+}$  inside the neurons [48]. Such a mechanism could lead to  $\text{Ca}^{2+}$   
542overload of neurons potentially contributing to neurotoxicity in our  
543model.

544 Dendritic tree of each PC has thousands of synaptic connections  
545with parallel fibers and 1-2 synapses with climbing fibers [49]. Hence  
546abnormality of dendritic morphology could lead to alteration of basic  
547synaptic transmission, such as EPSC. Our data suggest that S100 $\beta$  did  
548not affect presynaptic glutamate release because it did not significantly  
549change PF EPSC amplitude (Sup. Fig. 2A) and PPF ratio (Fig. 5A).  
550Interestingly, in PCs of 3 week-old SCA1 KI mice, where Ataxin-1 in  
551ubiquitously express, PPF ratio was increased in comparison to its WT  
552littermates (Fig. 6A). We suspect than this phenomenon is due to  
553slowing of glutamate-glutamine cycle machinery because application of  
554FC leads to the same effect in PBS and S100 $\beta$  injected PCs (Fig. 8C).  
555We also show that PF-EPSC rise time is increased in 3 weeks old SCA1  
556KI mice (Fig. 6B). It is notable that in B05 mice that express mutant  
557Ataxin 1 selectively in PCs rise time was not changed at the same age  
558[50]. Therefore it is likely that changes caused by Ataxin 1 in other cells  
559such as Muller glia are responsible for this effect. Injections of S100 $\beta$   
560accelerated PF EPSCs decay time (Fig. 5C) which also points to the  
561involvement of the Bergmann glia, which plays the key role in  
562glutamate uptake [51, 52]. Application of TBOA which blocks this  
563uptake, ameliorated the differences between PF-EPSC decay time of  
564PCs in PBS and S100 $\beta$  areas, consistent with this hypothesis (Fig. 9B).

565 At the same time, astrogliosis and neurodegeneration is  
566characterized by suppression of EAAT1 and EAAT2 function and  
567accumulation of extracellular glutamate leading to excitotoxicity [52]. It  
568highly likely that with age the tendency for prolongation of PF-EPSC  
569decay time SCA1 KI mice (Fig. 6C) will increase and become significant.

570Therefore we acknowledge that our S100 $\beta$  injection model may not fully  
571reflect the complex pathological process in Bergmann glia.

572 Effect of S100 $\beta$  on short-term synaptic plasticity was assessed using  
573DSE. In DSE, strong depolarization leads to Ca<sup>2+</sup>-dependent release of  
574endocannabinoids from PCs, which retrogradely activate the CB1  
575receptors on the terminals of PFs [53]. Activation of CB1 inhibits  
576glutamate vesicular release. S100 $\beta$  did not alter PPF ratio which  
577suggests that as such the vesicular release machinery remained intact  
578(Fig. 5A). It is acknowledged that CB may also have direct effects on  
579glia [54] but obviously under our conditions we did not reveal this  
580component.

581 Our modelling also confirmed that while DSE protocol  
582depolarization was sufficient to decrease PF-EPSC amplitude up to 67-  
58369% of control in S100 $\beta$  and PBS injected mice (Fig. 7A) there was no  
584difference between these two groups. Thus, the induction phase of DSE  
585was not affected by S100 but the recovery after the initial depression  
586was much faster in S100 $\beta$  injected slices. In PBS injected animals  
587amplitude of PF-EPSC was fully restored to the control level at ~100  
588sec after depolarization, but it only took 60-70 sec in S100 $\beta$  injected  
589group. The predicted speed of restoration by the double waveform  
590fitting (shown by solid and dashed lines on Fig.7A) resulted in the  
591significantly shorter recovery half-time in S100 $\beta$  group compared to the  
592PBS. Endocannabinoids are degraded by fatty acidamide hydrolase and  
593monoacylglycerol lipase. Faster recovery from DSE could be a result of  
594upregulation of these enzymes or simply indicate that S100 $\beta$  affected  
595the process of endocannabinoid release. A possible explanation for the  
596increased PF-EPSC recovery is an increase in extracellular glutamate  
597concentration. In astrocytes CB receptors acts as antagonists of  
598neuronal CB1 receptors and facilitate the neurotransmitter release [54,  
59941]. Possibly, S100 $\beta$ -activated astrocytes release more glutamate to  
600synaptic cleft after their CB receptors activation by DSE. In such We  
601used FC to evoke metabolic “starvation” of astrocytes. Interestingly  
602DSE (Fig. 8E) in PBS injected areas was sensitive to FC but in S100 $\beta$  it  
603was not. This suggests that the reactive astrocytes after S100 $\beta$  injection

do not respond to endocannabinoids. These data correlates with previous findings [54, 55].

In summary, elevated extracellular S100 $\beta$  leads to reorganization of glia/neuron morphology and disturbs synaptic transmission. Our findings are reminiscent of the early stage of a neurodegenerative process in cerebellar cortex such as seen from 3 old weeks SCA1 mice [25]. The changes in PF-EPSC kinetics reported here were not seen in non-cell selective SCA1 model mice, but take place in PC selective SCA1 model mice, where astrocytes are also affected by the mutant Atxn1 [24 and Fig. 6B]. We hope that our model will assist in better understanding of the role of glia in SCA1 and other diseases which affect cerebellum.

## **Figure Legends**

### **Figure 1. Localization of S100 $\beta$ in mouse cerebellum.**

(A) Confocal image of a cerebellar slice injected with PBS. Left: anti-S100 $\beta$ . Right: anti-GFAP. Scale bar 1mm.

(B) High power confocal image of the zone indicated on (A). Scale bar 100 $\mu$ m.

(C) and (D) - as above but from mice pre-injected with S100 $\beta$ . Scale bar in (C) is 1mm, in (D) 100 $\mu$ m.

**Figure 2. S100 $\beta$  alters morphology of Bergmann glia.** Microphotographs show anti-S100 $\beta$  and anti-GFAP immunoreactivity in

634 injected areas from PBS (A) and S100 $\beta$  (B) treated mice. Scale bar  
 635 50 $\mu$ m. (C) Summary graph showing the thickness of Bergmann glia  
 636 processes in  $\mu$ m. \*\*\*  $p < 0.001$  (D) In a comparison to PBS injected  
 637 areas, there were significantly fewer Bergmann glia processes per 100 $\mu$   
 638 in S100 $\beta$  injected areas. \*  $p < 0.05$  (E) Bergmann glia's processes were  
 639 more sparse in S100 $\beta$  injected areas. \*  $p < 0.05$  (F) Reduction in  
 640 Bergmann glia cell bodies caused by S100 $\beta$ . \*\*  $p < 0.01$

641

642 **Figure 3. Analysis of Bergmann glia process morphology in**  
 643 **PBS and S100 $\beta$ -injected mice.** (A) White and black  
 644 microphotographs of anti-GFAP-labeled areas of cerebellar cortex  
 645 injected with PBS (left images) and S100 $\beta$  (right images). Light images  
 646 contain digitally traced Bergmann glia processes generated using  
 647 ImageJ software. Arrows show the proximal processes which were  
 648 found more often in S100 $\beta$  injected areas. Scale bar 50 $\mu$ m. (B)  
 649 Maximum number of processes per Bergmann glia cell did not change  
 650 between PBS and S100 $\beta$ -injected areas. (C) Sholl analysis for PBS-  
 651 (open circles) and S100 $\beta$ -injected (closed circles) Bergmann glia cells  
 652 (number of intersections per 10 $\mu$ m of processes length). \*\*  $p < 0.01$ ,  
 653 \*\*\*  $p < 0.001$

654 **Figure 4. S100 $\beta$  alters the PCs morphology of mouse**  
 655 **cerebellum.** (A) Comparison of immunoreactivity to anti-  
 656 calbindin (PCs marker), and anti-GFAP in injected areas with  
 657 PBS (upper panel) and S100 $\beta$  (lower panel). The molecular layer  
 658 selected by white broken lines. Scale bar 50 $\mu$ m. (B) In  
 659 comparison to PBS injected areas, the molecular layer was  
 660 significantly thinner (79,6%) in S100 $\beta$  injected areas. (C) In a  
 661 comparison to PBS injected areas the capacitance of PCs  
 662 dendrites and soma measured by voltage-clamp was significantly  
 663 smaller in S100 $\beta$  injected areas. \*\*  $p < 0.01$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

664 **Figure 5. S100 $\beta$  alters PCs electrophysiological**  
 665 **properties** (A) The summary graph shows the average PFF ratio  
 666 (2nd amplitude /1st amplitude EPSC) in PCs from PBS and S100 $\beta$

injected areas, no significant differences found. Below -  
representative traces of PF-EPSCs (B) The summary graph  
shows the average rise time of PF-EPSCs in PCs from PBS and  
S100 $\beta$  injected areas. In comparison to PBS injected areas, rise  
time was significantly longer in S100 $\beta$  injected areas.  
Representative traces of PF-EPSCs are shown above. (C) In  
comparison to PBS injected areas, the decay time was  
significantly longer in S100 $\beta$  injected areas. The represented  
traces of PF-EPSCs are above.\*  $p < 0.05$ .

**Figure 6.** Electrophysiological properties of PCs are altered in 3  
weeks old non-cell selective SCA1 model mice. (A) Average PPF  
ratio (2nd amplitude /1st amplitude EPSC) in PCs from WT and  
SCA1 KI mice. In comparison to WT PCs, the PPF ratio  
significantly increased in SCA1 KI mice. Above representative  
traces of PF-EPSCs. (B) The average rise time of PF-EPSCs in  
PCs from WT and SCA1 KI mice. In comparison to WT PCs, the  
rise time was significantly longer in SCA1 KI animals.  
Representative traces of PF-EPSCs are above. (C) Average decay  
time of PF-EPSCs in PCs from PBS and S100 $\beta$  injected areas. No  
significant differences. The represented traces of PF-EPSCs are  
above. \*  $p < 0.05$ .

**Figure 7.** S100 $\beta$  disrupts the DSE at PF-PC synapses. (A)  
Average time course diagram of PF-EPSC amplitudes before and  
after depolarization. The amplitudes of PF-EPSC were  
normalized to values before depolarization. The numbers (n) of  
tested PCs and animals (PCs/animals) are indicated in the graph.  
Dotted and black lines indicate the double waveform model fit  
for PBS and S100 $\beta$  injected groups, respectively. Above -  
representative PF-EPSC traces from PCs from PBS and S100 $\beta$ -  
injected mice are above the diagram. Time points: before (1) and  
50 sec after (2) depolarization. (B) PF-EPSC amplitudes 50 sec  
after depolarization. In comparison to PBS injected areas, the

699 PF-EPSC amplitude was significantly bigger in S100 $\beta$  injected  
700 areas.\*  $p < 0.05$ .

701 **Figure 8. Downregulation of astrocytic function by FC**  
702 **affects amplitude and kinetics of PF-EPSC in PF-PC**  
703 **synapses.** (A) Time course of PF-EPSC amplitudes before and  
704 after 50 $\mu$ M FC application. (B) Averaged PF-EPSC amplitudes 10  
705 min after FC application. Effect of FC was approximately the  
706 same in S100 $\beta$  ad PBS injected slices. (C) FC changed  
707 significantly PF-EPSC rise time in PBS-injected animals, and  
708 slightly increased it after administration of S100 $\beta$  \*\*  $p < 0.01$ , †  
709  $p < 0.05$ . (D) Representative PF-EPSC traces elicited in PCs from  
710 PBS and S100 $\beta$ -injected mice are above the diagram. Time  
711 points: before (1) and 50 sec after (2) depolarization. (E) PF-  
712 EPSC amplitudes 50 sec after depolarization. In comparison to  
713 PBS injected areas, the PF-EPSC amplitude was significantly  
714 bigger in S100 $\beta$  injected areas.\*  $p < 0.05$  Bergmann glia  
715 suppression by FC leads to significant DSE enchantment in PBS  
716 injected PCs. † $p < 0.05$ . (F) Average time course of PF-EPSCs  
717 before and after depolarization in slices treated with 50 $\mu$ M FC.  
718 Dotted and black lines indicate the double waveform model fit  
719 for PBS and S100 $\beta$  injected groups, respectively.

720

721

722 **Figure 9. Inhibition of glutamate uptake by TBOA alters PF-EPSC**  
723 **kinetic but does not affect endocannabinoid-mediated short term**  
724 **plasticity.**

725

726 (B) (A) TBOA prolongs EPSP in PBS and S100 $\beta$ -injected tissues (\*  
727  $p < 0.05$ ). Note that the baseline tau was reduced after S100 $\beta$   
728 application but the effect of TBOA was comparable to control.  
729 TBOA had minimal effect on PPF ratio only in slices from  
730 S100 $\beta$  injected animals. \*  $p < 0.05$ .



(C) Average diagram of PF-EPSC amplitudes before and 30 sec after depolarization. Application of TBOA marked by red line. The amplitudes of PF-EPSC were normalized to values before depolarization.\*  $p < 0.05$ . (D) Representative PF-EPSC traces elicited in PCs from PBS and S100 $\beta$ -injected mice are above the diagram. Time points: before (1) and 30 sec after (2) depolarization. Average time course of PF-EPSCs before and after depolarization in slices treated with TBOA. Dotted and black lines indicate the double waveform model fit for *PBS* and S100 $\beta$  injected groups, respectively.

#### **Supplementary Materials:**

Supplementary Figure 1. (A) Confocal image of S100 $\beta$ -injected area (anti-S100 $\beta$  and anti-GFAP staining). The line with two arrowheads illustrates the 100 $\mu$ m length where the parameters of Bergman glia processes were evaluated (B) Confocal image to illustrate staining with anti-Calbindin and anti-GFAP antibodies. The line with two arrowheads illustrates the thickness of cerebellar molecular layer.

Figure S2: Electrophysiological characteristics of PCs (A) The summary graph shows the average PF-EPSC amplitudes in PCs from PBS and S100 $\beta$  injected areas. There was no significant differences between the two groups. The numbers (n) of tested PCs and animals (PCs/animals) are indicated in the graph. (B) Average PF-EPSC amplitudes in PCs from 3 weeks old WT and SAC1 KI mice. Differences between the groups were not significant. The numbers (n) of tested PCs and animals (PCs/animals) are indicated in the graph.

Figure S3: PBS injections do not alter decay time of PF-EPSC.

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#### 768**Author contributions**

769A.N.S., M.V.S., E.A.P. designed research. A.N.S. performed S100 $\beta$  injections., A.N.S.,  
770O.S.B performed the electrophysiological experiments, the morphological analysis. D.A.Y.  
771performed the immunohistochemistry, I.V.P., A.V. performed the Sholl analysis, A.N.S.,  
772analysed the data and performed statistics. A.N.S. wrote the paper. All authors approved the  
773final version of the manuscript.

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